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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/306,986
Filing Date: May 7, 1999
Appellant(s): Trinh et al.

Natalie Davis
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 10/24/2006 appealing from the Office action mailed 7/25/2006.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

Joseph G. Major, Biotechniques, Vol 12, No. 1, 1992, pages 40-43.

Deana and Belasco, Mol. Microbiology, Vol. 51, No. 4, pp 1205-1217, 2004.

Maudru et al., Journal of Virological Methods 66: 247-261, July 1997.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claim 8-12, 56, 70-73 are rejected under 35 U.S.C. 102(b) as being anticipated by Major (Biotechniques 12:40-43, 1992) as evidenced by Deana and Belasco (Mol. Microbiology, Vol. 51, No. 4, pp 1205-1217, 2004).

Major teaches a rapid PCR method of screening for point mutations. The taught method involves ascertaining the presence of a desired mutation within the mutated fragment or within some vector into which the mutated fragment has been cloned. Major teaches a method which comprises the synthesis of a nucleic acid molecule from a preparation comprising RNA and double-stranded DNA, said method comprising mixing the preparation with one or more DNA polymerases and incubating said mixture under conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of a template nucleic acid molecule. The method taught by Major specifically involves the PCR amplification, using Taq DNA polymerase, of a DNA fragment from the expression plasmid, pBluescript 11 SK(+), either sampled directly from JM109 *E. coli* colonies or from a bacterial plasmid isolate. Major further teaches that some primers, especially those with a 3'-terminal T-'T mismatch result in extra minor bands when bacterial colony lysates were used for the starting material. This thus decreases the sensitivity of the taught assay. Major does not teach the inclusion of ribonuclease in

the taught method, however, the bacterial lysate mixture taught by Major et al. inherently comprises ribonuclease. The inclusion of ribonuclease in the mixture and thus method taught by Major is evidenced by Deana and Belasco (Mol. Microbiology, Vol 51 No. 4, pp 1205-1217, 2004) who teach that *E. coli* inherently comprise a number of RNases that are capable of degrading single stranded RNA. It is noted that the reference Deana and Belasco is not available as prior art, however, this is unnecessary as this reference is only used to evidence that which is inherent in that method taught by Major.

Thus claims 8-12, 56, 70-73 are anticipated by Major as evidenced by Deana and Belasco.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claim 8-12, 56, 70, 71 and 73 are rejected under 35 U.S.C. 103(a) as being unpatentable over Major and Maudru et al. (J. Virological Methods 66:247-261, 1997).

As stated above, Major teaches a rapid PCR method of screening for point mutations. The taught method involves ascertaining the presence of a desired mutation within the mutated fragment or within some vector into which the mutated fragment has been cloned. Major teaches a method which comprises the synthesis of a nucleic acid

molecule from a preparation comprising RNA and double-stranded DNA, said method comprising mixing the preparation with one or more DNA polymerases and incubating said mixture under conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of a template nucleic acid molecule. The method taught by Major specifically involves the PCR amplification, using *Taq* DNA polymerase, of a DNA fragment from the expression plasmid, pBluescript 11 SK(+), either sampled directly from JM109 *E. coli* colonies or from a bacterial plasmid isolate. Major further teaches that some primers, especially those with a 3'-terminal T:T mismatch result in extra minor bands when bacterial colony lysates were used for the starting material. This thus decreases the sensitivity of the taught assay. Major does not teach the inclusion of ribonuclease in the taught method of synthesizing nucleic acids.

Maudru et al. examine the cause and teach a method for the elimination of background signals in a modified polymerase chain reaction-based reverse transcriptase assay. Maudru et al. teach that the background signal of the PCR-based reverse transcriptase (PBRT) assay was due to an intrinsic RNA-dependent DNA polymerase activity of the Taq DNA polymerase enzyme used for the assay. They further teach that this background signal could be eliminated by inserting a ribonuclease digestion step prior to amplifying the cDNA product of the RT reaction by PCR. Thus Maudru et al. anticipates claims 8-12 drawn to a method for synthesizing a nucleic acid molecule said method comprising: a) mixing a nucleic acid template, with one or more DNA polymerases and one or more peptides or polypeptides having ribonuclease activity; and b) incubating said mixture under condition sufficient to synthesize a nucleic

acid molecule complementary to all or a portion of said template, wherein said ribonuclease activity is seleced from the group consisting of fragments, variants, derivatives or mutants of those RNAses listed in claim 9, wherein said thermostable DNA polymerase is selected from the group consisting of fragments, variants, derivatives or mutants of those thermostable DNA polymerases listed in claim 12.

One of ordinary skill in the art at the time of filing would have been motivated to add a polypeptide with ribonuclease activity to the method taught by Major, in order to remove residual RNA sequence contamination from the targeted nucleic acid template in any preparation which would contain substantial amounts of RNA, such as a bacterial colony lysate, in order to decrease the level of background signal from the taught PCR assay. As the ordinary artisan would know that any nucleic acid preparation that has not been purified, such as a bacterial colony lysate, contains substantial amounts of contaminating RNA, the motivation for the removal of these contaminating sequences is that this would increase the sensitivity of the taught PCR assay method from bacterial colony lysates, thus eliminating the need for purification of the template DNA and reducing time and work needed to perform the assay. This is supported by both Major, who teach that some primer sets when used with bacterial colony lysates result in extra minor bands, and Maudru et al. who teach that the background signals of PDR based nucleic acid synthesis reactions is due to an intrinsic RNA-dependent DNA polymerase activity of Taq DNA polymerase. The reasonable expectation of success for the inclusion of ribonuclease in the nucleic acid synthesis reaction of Major comes from the high degree of knowledge in the field of nucleic acid synthesis and the results of Maudru

et al. who teach that the simultaneous addition of ribonuclease in order to eliminate background signals in the polymerase chain reaction containing Taq DNA polymerase did not adversely affect the synthesis of the desired nucleic acid products by PCR.

Thus claims 8-12, 56, 70, 71 and 73 are made obvious by Major and Maudru et al.

(10) Response to Argument

Appellants traverse the current rejection of claims 8-12, 56 and 70-73 under 35 U.S.C. 102(b) as being anticipated by Major (Biotechniques 12:40-43, 1992) as evidenced by Deana and Belasco (Mol. Microbiology, Vol. 51, No. 4, pp 1205-1217, 2004).

After a review of the applicable law (i.e. 35 U.S.C. 102), the references used in the rejection and the basis of the rejection, appellants traverse the rejection on the following basis.

Appellants submit that the Major reference does not anticipate the currently pending claims on the basis that Major discloses PCR-based assays using either bacterial lysate or mini-prep DNA as starting material and contrary to the examiners position, appellants submit that the "clarified bacterial colony lysate" used in the assay of Major does not necessarily contain RNAses. Appellants submit that the Deana and Belasco reference does not support the Examiners inherent anticipation argument.

Appellants submit that even if the Examiner's inherent anticipation argument is correct, there is nothing in the Major reference that teaches mixing a preparation comprising RNA and double-stranded DNA with one or more DNA polymerases and

one or more peptides or polypeptides having ribonuclease activity, as specified by the currently pending claims.

Appellants submit that the Major reference discloses the addition of a DNA polymerase to a sample (i.e. clarified bacterial lysate) which appellant's state may (or may not) contain an RNase. Appellants submit that adding a DNA polymerase to a biological sample that already contains an RNase, where the RNase is derived from the cellular source of the sample, is clearly distinct from mixing the sample with a DNA polymerase and an RNase, as required by the present claims. Thus, regardless of whether E. coli inherently contains RNases, the Major reference does not teach a method that includes all of the steps that are encompassed by the currently presented claims.

Appellants appear to argue the current rejection based upon anticipation on two bases. First appellants question whether or not the "clarified bacterial lysate" used by Major for the taught PCR assays, contains RNases. Second, appellants then submit that even if the above "inherent anticipation" argument is correct, there is nothing in the Major reference that teaches mixing a preparation comprising RNA and double-stranded DNA with one or more DNA polymerases and one or more peptides or polypeptides having ribonuclease activity, as specified by the currently pending claims.

In response to appellant's first raised issue, whether the "clarified bacterial lysate" taught by Major contains RNases, let us first look at exactly what the "clarified bacterial lysate taught by Major is. As taught by Major,

> E. coli strain JM109 colonies containing pBluescript II SK(+) were either sampled directly for PCR or standard plasmid isolation from

> bacterial colonies was performed as follows: One medium-size colony was subjected to vigorous vortexing in 10ul of sterile, distilled water and boiled in a water bath for 10 min. The cellular debris was removed by centrifugation at room temperature for 5 min in a lab-top microcentrifuge.
>
> The taught PCR assay used 10ul of the above "clarified bacterial colony lysate"

As previously stated, Major does not teach the inclusion of additional ribonuclease in the taught method, however, the bacterial lysate mixture taught by Major et al. inherently comprises ribonuclease. The presence of ribonuclease in the mixture and thus method taught by Major is evidenced by Deana and Belasco (Mol. Microbiology, Vol 51 No. 4, pp 1205-1217, 2004) who teach that the bacteria *E. coli* inherently comprise a number of RNases that are capable of degrading single stranded RNA. Appellants appear not to question that which is evidenced by Deana and Belasco, that E.coli inherently comprise a number of RNases that are capable of degrading single stranded RNA. It would appear that appellants question is directed to whether or not any of these RNases are in the bacterial lysate mixture of Major that is used for the subsequent PCR assays. Prior to its use in the PCR assay of Major, the only manipulation of the RNase containing, E. coli strain JM109 bacterial cells is vigorous vortexing in 10 ul of distilled water and boiling for 10 minutes, followed by 5centrifugation for 5 minutes in a lab-top centrifuge. The vortexing and boiling of the bacterial lysate results in the lysis of the bacterial cells, and the minimal centrifugation step removes cellular debris such as membranes and those cells not sufficiently lysed. Following this procedure, those cellular proteins and DNA which normally occur in the bacterial cell remain in the supernatant of the centrifuged bacterial lysate. As RNAse is not a membrane bound protein, but is a soluble protein, it would remain in the

supernatant of the clarified E. coli JM109 bacterial lysate. Thus this RNAse that was in the JM109 E.coli bacterial cell, as evidenced by Deana and Belasco, is in the PCR assay mixture taught by Major. Thus the methods taught by Major anticipate the claims to a method for synthesizing a nucleic acid molecule from a preparation comprising RNA and double-stranded DNA, said method comprising: a) mixing the preparation with one or more DNA polymerases (i.e. Taq polymerase as taught by Major) and one or more peptides or polypeptides having ribonuclease activity (in the "*E. coli* clarified bacterial lysate" as discussed above) wherein said RNase is capable of degrading single-stranded RNA and b) incubating said mixture under "conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of said double-stranded DNA and under which said peptides or polypeptides having ribonuclease activity degrade said single strand-stranded RNA". Thus appellant's claims are anticipated by Major.

Appellants' second basis for traversal of the rejection under anticipation by Major, is that if we assume that the inherent anticipation argument is correct, as discussed above, then the teaching of Major is still deficient, since appellants submit that nothing in the Major reference teaches mixing a preparation comprising RNA and double-stranded DNA with one or more DNA polymerases and one or more peptides or polypeptides having ribonuclease activity, as specified by the currently pending claims.

Appellants submit that the Major reference discloses the addition of a DNA polymerase to a sample (i.e. clarified bacterial lysate) which appellant's state may (or may not) contain an RNase. Appellants submit that adding a DNA polymerase to a

biological sample that already contains an RNase, where the RNase is derived from the cellular source of the sample, is clearly distinct from mixing the sample with a DNA polymerase and an RNase, as required by the present claims. Thus, regardless of whether *E. coli* inherently contains RNases, the Major reference does not teach a method that includes all of the steps that are encompassed by the currently presented claims. An appellant argument is flawed in that the mere addition of the *Taq* DNA polymerase to the clarified bacterial lysate containing RNA, double-stranded DNA and RNase as taught by Major constitutes "mixing the preparation" with one or more DNA polymerases and RNases. Nothing in appellants' claimed method excludes that the RNase cannot inherently be a part of the preparation comprising RNA and double-stranded DNA. There is no method step which requires that an RNase which is external to the preparation be added to the preparation.

Thus for the reasons of record and for those repeated herein claims 8-12, 56, 70-73 remain rejected under 35 U.S.C. 102(b) as being anticipated by Major (Biotechniques 12:40-43, 1992) as evidenced by Deana and Belasco (Mol. Microbiology, Vol. 51, No. 4, pp 1205-1217, 2004).

Appellants traverse the current rejection of claims 8-12, 56 and 70, 71 and 73 under 35 U.S.C. 103(a) as being unpatentable over Major (Biotechniques, Vol 12, No. 1, 1992, pages 40-43) and Maudru et al. (Journal of Virological Methods 66: 247-261, July 1997).

After a review of the applicable law (i.e. 35 U.S.C. 103), the references used in the rejection and the basis of the rejection, appellants traverse the rejection on the following basis.

Appellants submit claims 8-12, 56, 70, 71 and 73 are not obvious over Major and Maudru et al.

Appellants continue to traverse the current rejection on the following basis. Appellants refer to the statements in the previous office action.

> While Major does not attribute background difficulties to contaminating RNA, one of skill in the art would realize that given the employment of the method of Major to bacterial lysates, there would be a substantial amount of background RNA in the preparation. This knowledge in combination with that taught by Maudru et al. stating that the background signal in a similar assay was found to be due to an intrinsic RNA-dependent DNA polymerase activity of the *Taq* DNA polymerase, would lead one of skill in the art who was attempting to successfully use a PCR method to screen for small mutations to include a ribonuclease digestion step prior to PCR amplification, as a means of making the assay more sensitive. In support of the above, applicants attention is drawn to Major, page 42, middle column, which states "the present results indicate that all three possible terminal T mismatches can be equally discriminated under standard PCR conditions, **especially** when using mini-prep DNA". Such a statement clearly supports that even Major recognized the taught method had different results or sensitivities depending on the template used (noting the reference to "especially"), although Major did not comment on the specific difference of the two different types of template preparations. One of skill in the art would understand that the difference was likely the presence of contaminating material, such as RNA.

Appellants continue to argue the rejection on the basis that the position previously taken that one of skill in the art would have attributed the "Extra Minor Bands" mentioned in the Major reference to the presence of contaminating RNA in the reactions is incorrect. Appellants submit that Major did not attribute these extra minor bands to

contaminating RNA, but rather to amplification products produced from oligonucleotides that have 3'-terminal nucleotide mismatches.

Appellants submit that the above reasoning is both logically and technically flawed based on the following:

Appellants continue to submit that a bacterial lysate contains many factors besides RNA (e.g., proteins, salts, lipids, signaling molecules, etc.) and that these other factors are also absent from mini-prep DNA, and thus there is no reason why one of ordinary skill in the art would have specifically regarded RNA as the one factor responsible for the difference in 3'-terminal mismatch discrimination alluded to in Major. Appellants submit that the only such evidence is presented in appellant's own specification, which can not be used against appellants. It is noted to appellants that it remains whether or not RNA is the "one factor" responsible for the difference in 3'-terminal mismatch discrimination, alluded to in Major, however, it is believed that the presence of RNA is a major difference between plasmid DNA and bacterial lysates and thus a contributing factor. Appellants above point has again been considered in full, however, found non-persuasive. As previously stated in the original rejection, Maudru et al. teach that the background signal of the PCR-based reverse transcriptase assay is due to an intrinsic **RNA-dependent** DNA polymerase activity of the *Taq* DNA polymerase enzyme and they teach that this background signal could be eliminated by the addition of a ribonuclease to the amplification reaction".

This previously presented evidence as taught by Maudru, (not appellants own specification) is in part the basis of the conclusion that of the components present in a

bacterial lysate relative to a mini-prep, it is the RNA, which is most likely the source of interference.

Appellants' statement that "the examiner's implication that a person of ordinary skill in the art would have believed that errors in terminal nucleotide mismatch discrimination occur only in bacterial colony lysates (which contain RNA) but not in other DNA preparations (which do not contain RNA) is incorrect" is confusing, as the examiner is unaware of any such statement or implication made during prosecution of this application. Further such statements would seem to be irrelevant to the logic upon which the current rejection is made. As previously stated, Major teaches that some primers, especially those with a 3'-terminal T:T mismatch result in extra minor bands when bacterial colony lysates were used for the starting material. This is opposed to starting material which was mini-prep DNA.

Appellants' reference to Kwok et al. continues to be acknowledged, however, continues to be found not persuasive, because it is irrelevant to the taught amplification of bacterial lysates. Appellants' discussion relative to Kwok et al. of assay systems involving the use of plasmid DNA or PCR-generated products as templates is irrelevant to errors observed between bacterial lysates and mini-prep DNA. Similarly the presentation of the reference Charlieu is not considered relevant to the methods taught using bacterial lysates.

Secondly, appellants assert that a person of ordinary skill in the art would not have had any motivation to combine Maudru with Major as the references deal with entirely different non-analogous assay systems. Appellants' argument is

acknowledged, however, found nonpersuasive, because the basis to combine the references is as previously stated, based on the fact that both references teach methods of amplification of nucleic acids. Appellant's characterization of Maudru as being concerned with assaying the presence of reverse transcriptase in a sample is misleading. In support, appellant's attention is directed to the title of Maudru which is "Elimination of background signals in a modified polymerase chain reaction-based reverse transcriptase assay".

Appellants complete argument is acknowledged, however continues to be found non-persuasive and the rejection of claims 8-12, 56, 70, 71 and 73 is maintained for the reason previously made of record and repeated above.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

Richard Hutson

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